REMARKS

Claims 19-24 and 26-34 are pending. Claim 18 has been canceled.

Claims 22 and 28 have been amended to correct a minor grammatical error. Claim 28 has been amended to correct a typographic error. This amendments are believed to overcome the claim objections and do not alter the scope of the claims.

New claims 29 and 32 - 34 are supported on page 16, lines 27 - 29 and throughout the examples. New claims 30 and 31 are supported on page 3, lines 24 to 29.

This amendment is further supported throughout the specification. No new matter is added by this amendment.

Drawing

The examiner has noted that the drawing sheet for figure 13 does not have a label.

A corrected drawing sheet is being provided herewith.

Rejection Under 35 USC §112, Second Paragraph.

Claims 18 and 19 have been rejected under 35 USC §112, second paragraph, as lacking antecedent basis for "the rAAV".

Claims 22-24 and 28 have been rejected under 35 USC §112, second paragraph for the use of the term "genomes of rAAV". The examiner suggests that claims 21 and 26 be amended to provide antecedent basis of "rAAV" and claims 22-24 and 28 be amended to recite "the rAAV".

Claims 21 and 26 have been rejected under 35 USC §112, second paragraph, for the use of the phrase "wherein the level of contaminating adenoviral helper virus is not greater than that obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation".

Claim 27 has been rejected under 35 USC §112, second paragraph, for reciting the phrase "wherein the ApoE is administered intramuscularly", when the method of claim 26 recites administration of a recombinant AAV vector, not ApoE.

The claims have been amended to address these issues relating to antecedent basis. This amendment does not alter the scope of the claims.

No new matter is added.

The Invention

The observation by the inventors that delivery of rAAV which is at least as free of contaminating adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation (and particularly, skeletal muscle) leads to highly efficient transduction of muscle fibers leading to stable and prolonged expression of transgene, which led to the present invention. Thus, it is the inventors' observation which enabled the present invention which delivers ApoE and achieves prolonged expression of ApoE which is secreted and thus, found in the circulation for a prolonged period of time. Thus, the method of the invention is particularly well suited for delivery of ApoE for treatment of atherosclerosis in that it provides for prolonged expression of ApoE in the circulation and avoids a destructive immune response against the transduced cell expressing the ApoE. See, e.g., Example 6.

Further, this observation led to the finding that rAAV must be purified of helper adenovirus. This was essential to the present invention.

Further, having recognized and taught that a *minimum* level of purity of the rAAV was necessary, the inventors should not be limited to only the method of purifying away helper virus exemplified in the application. Nor should the inventors be limited to only that minimum level of purity which is taught to be necessary for the invention.

Rejection Under 35 USC §112, first paragraph

> Claims 18-24 and 26-28 have been rejected under 35 USC §112, first paragraph. The examiner argues that the specification lacks sufficient working examples to support claims which he states encompass rAAV vectors wherein the ApoE encoding sequences are not linked to a promoter or wherein multiple ITRs or multiple ApoE encoding sequences are present or wherein the levels of contaminating adenoviral helper virus are lower than the levels of contaminating adenoviral helper virus after subjecting the rAAV to four rounds of cesium chloride centrifugation or wherein the vector is administered by any method.

Applicants respectfully traverse this rejection.

The claims have been amended to clarify that the ApoE encoding sequences are linked to regulatory sequences which direct their expression in a cell transduced with the rAAV carrying the ApoE encoding sequences.

The claims do not recite multiple ITRs or multiple ApoE encoding sequences. Nor are applicants required to provide a specific working example for each embodiment encompassed by a claim. This is particularly true for such aspects of each embodiment as would be within the ability of one of skill in the art to make and use.

The claims have been amended to address the §112, second paragraph, objections relating to the phrase used to describe the levels of contaminating helper virus. Applicants believe that this amendment renders the above rejection moot with respect to this language.

Applicants have additionally added claims drawn to the subject matter which the examiner has stated is enabled:

> "A composition comprising a recombinant adeno-associated virus (AAV), wherein the rAAV comprises (i) a 5' AAV inverted terminal repeat (ITR), (ii) a nucleic acid sequence encoding human apolipoprotein E operably linked to a eukaryotic promoter, and (iii) a 3' ITR, and wherein the level of contaminating adenoviral helper virus is the same as that obtained by subjecting said recombinant AAV to four rounds of cesium chloride centrifugation.
>
> A method of delivering ApoE to a patient in need of treatment of atherosclerosis, wherein the method comprises the step of administering to the patient intramuscularly the composition comprising the rAAV and wherein the ApoE encoding sequence in the composition is expressed in the patient and wherein a cytotoxic immune response directed against rAAV-transduced cells of the patient expressing ApoE is absent in the patient."

Applicants request reconsideration of this rejection, and favorable consideration of the new claims.

Double -Patenting

> The double-patenting rejection over issued US Patent 5,866,522 has been maintained. Double-patenting rejections over co-pending US Application No. 09/757,673 and US application No. 09/237,064 have been raised.

Applicants respectfully request that the rejection be deferred until such time as the claims are otherwise in condition for allowance.

Rejections under 35 USC §103

> Claims 18-24 and 26-28 have been rejected under 35 USC §103(a) as being unpatentable over Podsakoff et al, US Patent 5,858,351, taken in view of Kashyap et al, J. Clin. Invest., 96:1612-1610 (September 1995). The examiner states that the prior art rejection is based, to paraphrase, entirely on the construction of the rAAV and not with regard to the intended use of the vector *in vivo*.

Applicants traverse this rejection with respect to both the method and composition claims.

The examiner is continuing to apply the same rejections against the method of delivery claims and the steps recited therein as against the composition claims. The examiner's statement appears to equate the recited method steps to no more than a recitation of an intended use in the preamble of claim drawn to the vector *in vivo*. This is improper.

With respect to the method claims, the deficiencies of the combination of these documents are several. Podsakoff contains no teaching or suggestion of the use of ApoE. Kashyap refer to intravenous infusion of a recombinant adenovirus containing human apolipoprotein E (apoE) in apoE-deficient mice.

This combination fails to provide the necessary suggestion that ApoE could be delivered by any route other than by intravenous delivery, or via another vector and also fails to provide a reasonable expectation of success. Neither of these documents recognizes the problems associated with the immune response generated to contamination of rAAV preparations with adenoviral helper virus.

The observation by the inventors that delivery of rAAV which is as free of contaminating adenoviral helper virus as is obtained by subjecting said recombinant

AAV to four rounds of cesium chloride gradient centrifugation (and particularly, delivery to skeletal muscle) leads to highly efficient transduction of muscle fibers leading to stable and prolonged expression of transgene led to the present invention. Thus, it is the inventors' observation which enabled the present invention which delivers ApoE and achieves prolonged expression of ApoE which is secreted and thus, found in the circulation for a prolonged period of time. Thus, the method of the invention is particularly well suited for delivery of ApoE for treatment of atherosclerosis in that it provides for prolonged expression of ApoE in the circulation and avoids a destructive immune response. See, e.g., Example 6.

In addition, a further claim has been added which recites the step of "monitoring the mammal for expression of ApoE". Applicants believe that this will aid in distinguishing the issues relating to patentability of the method from those of the composition.

Applicant request reconsideration and withdrawal of this rejection with respect to the method claims.

With respect to the composition claims, this combination of references is deficient. More particularly, the claims of the invention recite a composition comprising a rAAV carrying the ApoE transgene and a physiologically compatible carrier. Thus, such a *composition* is for delivery to a mammal, and particularly to a patient, and any rejection thereof can not be based entirely on the method of constructing an rAAV vector, as neither such a method nor the rAAV produced thereby are claimed. Absent motivation to deliver ApoE via a rAAV vector *and* a reasonable expectation of success, there can be no motivation to make a composition whose only purpose would be for *in vivo* delivery.

For the reasons discussed herein and in the prior papers, the combined teachings of Podsakoff and Kashyap fail to provide the necessary motivation and reasonable expectation of success for such a composition.

> The examiner points out that Podsakoff teaches that the purified rAAV isolates bands of average density of about 1.38 g/mL and the present specification described AAV particles having a density of 1.37-1.40 g/mL. The examiner argues that these passages are evidence that the rAAV purified by four rounds of CsCl centrifugation are no more free of contamination that the rAAV produced by Podsakoff.

This is not an accurate assessment of the comments in Podsakoff. It is known in the art, as evidenced by the teachings in the present specification, that rAAV has a density in the range of 1.37 - 1.40 g/mL. The teaching in Podsakoff does not provide evidence that its preparations are as free of contamination with helper as the rAAV of the present invention, because even if contaminated with Ad helper, the rAAV would band at the same density. In fact, Podsakoff recognizes the continued presence of Ad helper in his preparations, because he further treats his so-called "purified rAAV" to heat inactivate the helper Ad. While this inactivation may eliminate the biological function of the helper Ad, it is well known to those of skill in art that an inactivated virus is capable of inducing an immune response. Elimination of an immune response to the helper Ad is the very advantage provided by the present invention and which is lacking in the cited combination of prior art.

Applicants request reconsideration and withdrawal of this rejection with respect to the composition claims.

Attached hereto is a "**Version With Markings to Show Changes Made**" (Appendix A) and a "**Clean Copy of Pending Claims Without Markings**" (Appendix B).

The Director of the U. S. Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,

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Appendix A
Version with Markings to Show Changes Made

19(Twice Amended). The composition according to claim 21, wherein the recombinant [r]AAV [further] comprises a constitutive promoter.

21(Four Times Amended). A composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier,

wherein said recombinant AAV comprises (a) 5' AAV inverted terminal repeats (ITRs), (b) nucleic acid sequences encoding human apolipoprotein E (ApoE) operably linked to regulatory sequences which direct its expression, and (c) 3' AAV ITRs, and

wherein the [level of] recombinant AAV is at least as free of contaminating adenoviral helper virus [is no greater than that] as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation.

22(Amended). The composition according to claim 21, wherein said composition comprises at least 10^9 particles of recombinant [r]AAV.

23(Amended). The composition according to claim 21, wherein the composition comprises 2.5×10^{10} to 5×10^{10} genomes of recombinant [r]AAV.

24(Amended). The composition according to claim 21, wherein the composition comprises 5×10^{10} to 5×10^{11} genomes of recombinant [r]AAV.

26(Amended). A method of delivering apolipoprotein E ([a]ApoE) to a [patient] mammal with [in need of treatment of] atherosclerosis, said method comprising the step of

administering to the [patient] mammal a composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier,

wherein said recombinant AAV comprises (a) 5' AAV inverted terminal repeats (ITRs), (b) nucleic acid sequences encoding human apoliprotein E (ApoE) operably linked to regulatory sequences which direct expression thereof and (c) 3' AAV ITRs,

wherein the [level of] recombinant AAV is at least as free of contaminating adenoviral helper virus [is no greater than that] as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation,

and wherein the ApoE in said composition is expressed in the mammal [patient in the absence of a cytotoxic immune response directed against recombinant AAV-transduced cells expressing the ApoE].

27(Amended). The method according to claim 26, wherein [the apoE] said recombinant AAV is administered intramuscularly.

28(Amended). The method according to claim 26, wherein said composition comprises at least 10^9 genomes of recombinant [r]AAV.

Construction of AV.CMV ApoE

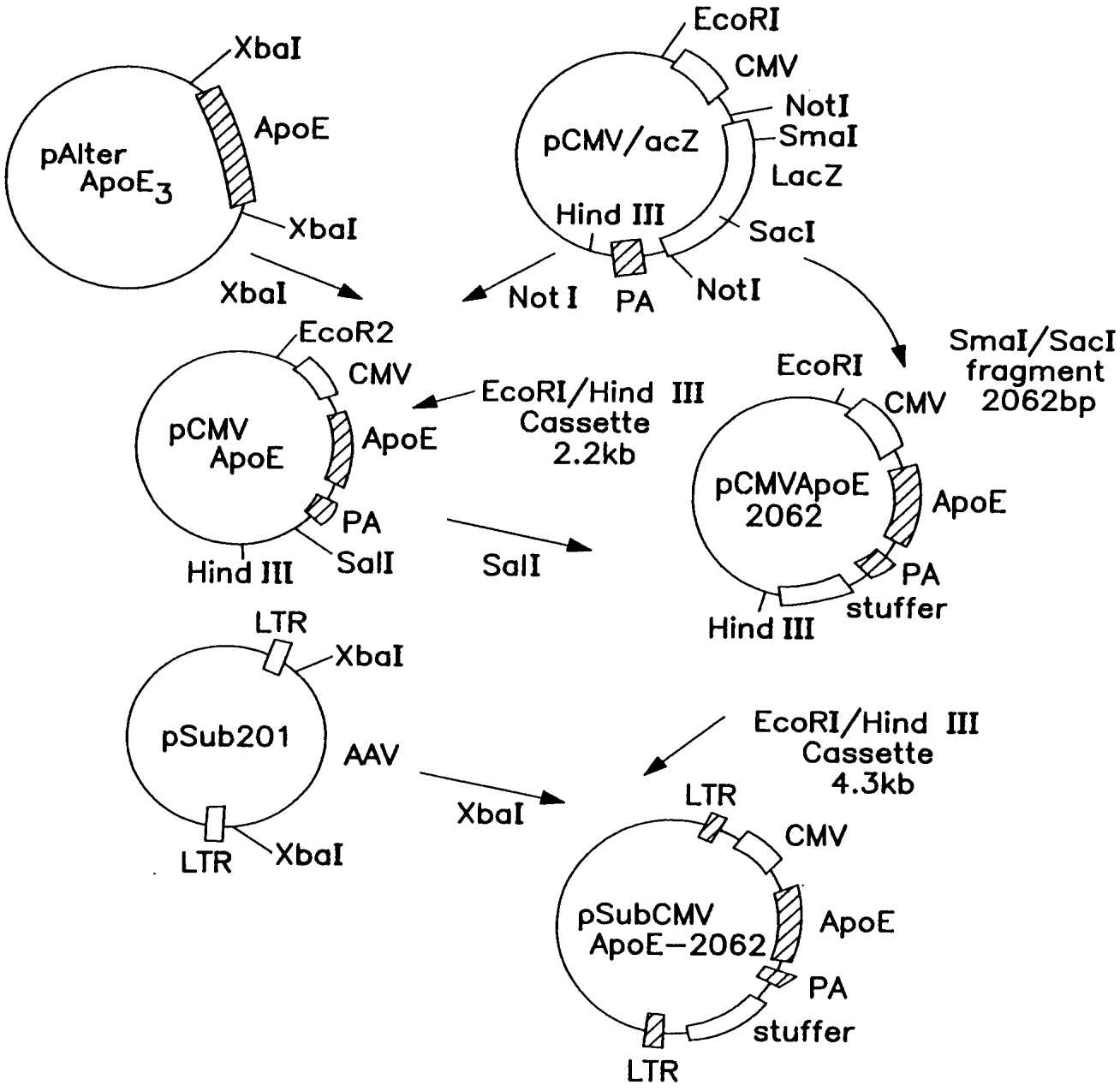

FIG. 13